

Grant Proposal

Bioprocesses for Metabolite Production from Marine Invertebrates: The European Horizon BLUES project

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Abstract

One of the major accomplishments of the 20th century is the advancement of pharmaceuticals to treat a variety of diseases and metabolic disorders. Traditionally, nature has served as the primary source for new pharmaceuticals, with over 50% of marketed drugs either derived directly from natural sources or synthesised using natural products as templates or starting materials. The ocean is home to more than 200,000 described species of marine invertebrates, representing every phylum, including twelve phyla that are exclusively marine. More than 15,000 novel marine-derived chemicals have been reported, many of which have potential pharmaceutical applications. Many of these compounds occur in marine organisms at very low concentrations and their often

complex molecular structures make them difficult to be chemically synthesised, which has created a bottleneck in their development as drugs. Scientists have spent decades, without success, working to develop alternative supply options, including *in vitro* culturing of marine invertebrates or cell cultures. The objective of BLUES is to expand the potential to produce valuable and unique bioactive compounds from marine invertebrates by developing novel *in vitro* cell culture systems for four phyla of marine invertebrates (Porifera, Cnidaria, Echinodermata, Chordata) and optimising production yields as an alternative to wild harvesting and chemical synthesis. The goal is to design a pathway towards industrial bioprocesses using cell lines as a chassis to produce unique, high-value, marine bio-based compounds. The novel bioprocesses will solve the supply bottleneck for increased availability of bioactive compounds, but also for a higher level of sustainable alternatives, contributing to the development of circular processing and a circular economy.

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Keywords

Biotechnology, sustainability, cell line development, bioprocess development, marine invertebrates, bioactive secondary metabolites, circular economy, hybridoma, deep eutectic solvents, 3D cell culture, scaffold materials/matrices

Introduction

The oceans cover over 70% of Earth's surface and 95% of Earth's biosphere and contain a wealth of biological diversity with more than 200,000 described species of marine invertebrates and algae and millions of microbial species (United Nations 2018). This vast biological diversity is accompanied by an equally remarkable chemical diversity of secondary metabolites. One evolutionary explanation for this vast array of chemical compounds is that many marine invertebrates are sessile as adults and have evolved in habitats which are often more challenging, compared to their terrestrial counterparts. This has resulted in the evolution of novel metabolic pathways and the production of structurally diverse chemicals that have a wide variety of ecological roles (Paul et al. 2011, Puglisi et al. 2014, Puglisi et al. 2019, Carroll et al. 2020), including compounds to reduce predation, enhance larval settlement or reproduction, contribute to direct inter- and intraspecific competition, reduce palatability or nutrient uptake by predators, protect against parasites, viruses and pathogens, as well as regulate host microbiomes.

Such compounds are being exploited for potential industrial uses, such as pharmaceuticals, cosmetics, nutritional supplements, molecular probes, enzymes, anti-foulants, fine chemicals and agrichemicals (Rotter et al. 2021, Rotter et al. 2024). For example, the first marine (sponge)-derived products to become clinically available were the result of a modest research programme in the 1950s (Bergmann and Feeney 2002),

which led to the synthesis of the nucleosides vidarabine (Ara-A), an antiviral drug and cytarabine (Ara-C), an anti-cancer drug. Further efforts slowly developed over the next two decades and, in the 1980s, major initiatives in marine drug discovery were initiated. Several marine-derived molecules since then have become clinically available as pharmaceuticals (Rodrigues et al. 2019, Saeed et al. 2021). Specifically, from 1990–2019, more than 15,000 new natural products from marine invertebrates have been reported (Calado et al. 2022, Brück et al. 2023), but only a small percentage of these compounds has been studied for potential uses (Li et al. 2018), in part, because of the supply bottleneck. The most prolific sources are Porifera (47.2%) and Cnidaria (35.3%), with Echinodermata and Chordata contributing 7.7% and 6.1%, respectively (Jeon et al. 2015, Kobayashi and Ishibashi 2000, Carroll et al. 2022).

Marine organisms produce a wide range of bioactive metabolites with potential human health applications, including those that have cytotoxic, antioxidant and antimicrobial properties. Marine invertebrates share a surprisingly high level of homology with more developed metazoans (Gamulin et al. 1994, Schäcke et al. 1994). Genes encoding for toll-like receptors (Cetkovic et al. 2004, Rodrigues et al. 2019), src-type proteins (Cetkovic et al. 2004, Ma et al. 2014), Ras-like small GTPases (Cetkovic et al. 2007), Bcl-2 homology domains (Wiens et al. 2000), death domains (Müller et al. 2000, Wiens et al. 2000), tyrosine kinases (Muller et al. 1999), mortalin (an HSP70 member that induces senescence in immortalised mouse fibroblasts) (Ben-Hamo et al. 2018) and survivin (a regulator of proliferation and apoptosis) (Luthringer et al. 2010), have all been identified in lower invertebrate taxa, like sponges (phylum Porifera) and show significant homology to mammalian genes. Antimicrobial peptides (AMPs) have gained attention as natural compounds possessing biological activities and significant potential for medical applications. AMPs are crucial effectors of the innate immune system of marine organisms and regulate proliferative activities and protective processes. In marine invertebrates, humoral factors encompass AMPs and peptides dissolved in coelomic fluid or haemolymph (Guryanova et al. 2023). The need for more research, characterising antimicrobial compounds from echinoderms, for example, as potential sources of novel pharmacologically relevant compounds, has already identified. (Hanifaturahmah et al. 2024, Zhao et al. 2024, Atanassova et al. 2024).

Marine-derived bioactive compounds can also address the rising demand for alternative nutraceuticals (Eroldoğan et al. 2022), pharmaceuticals and cosmeceuticals (Rotter et al. 2024), as well as for new bio-based products (Rotter et al. 2021). Additionally, marine biomass can be utilised to create bulk commodities like biofuels and bioplastics (Tennakoon et al. 2023). The sustainable use of marine resources and development of biomolecules and polymers are key aspects of marine biotechnology.

Despite the vast potential for marine compounds to act as new pharmaceuticals, cosmetics, nutritional supplements, molecular probes, catalysts, anti-foulants, fine chemicals, agrichemicals and other innovative bioproducts, the major bottleneck to the development of this biotechnological sector remains the limited supply of raw materials. Harvesting wild organisms from the marine environment is not ecologically sustainable, as many of the novel marine compounds are found only in minute quantities and are

often only synthesised under special conditions (Brück et al. 2023). Furthermore, a wide range of bioactive compounds/chemicals cannot be reproduced through chemical synthesis, aquaculture or wild collections. The testing of a single drug may require several kilograms of a specific bioactive metabolite for preclinical and clinical stages, potentially demanding thousands of tonnes of fresh material (Calado et al. 2022). Clearly, such biomass quantities cannot be harvested from nature without risking the continued existence of the respective species, resulting in many promising marine-derived natural compounds that can no longer be pursued for product development.

The BLUES consortium has been assembled to tackle this research challenge regarding the development of cell cultures from marine invertebrates. It is comprised of experts in cell culture of the four taxa of invertebrates (Porifera, Cnidaria, Echinodermata, Urochordata); marine invertebrate-microbe symbiosis; product down-stream processing; process intensification; and communication, exploitation and dissemination (Fig. 1). The consortium consists of nine partners from eight countries (Table 1) and is funded by a Horizon Europe Circular Economy and Bioeconomy Sectors (HORIZON-CL6-2023-CIRCBIO-01) grant, project number 101134820. The 4-year project was initiated on 1 January 2024 and is coordinated by Wageningen University (WU).

Table 1.

BLUES consortium members.

Partner	Country
Wageningen University	Netherlands
Moreforskning AS	Norway
Israel Oceanographic and Limnological Research Limited	Israel
Matis OHF	Iceland
Universidade Do Minho	Portugal
Universita Degli Studi Di Genova	Italy
Geonardo Kornyezetvedelmi Terinformatikai Es Regionalis Projektfejleszto Korlatolt Feelossegu Tarsasag	Hungary
Cellex SRL	Italy
University of New South Wales	Australia

Scientific Challenges and BLUES Innovation for the Blue Bioeconomy

In vitro production, generating bioactive compounds through cell cultures, has been considered as a promising solution for the supply problem, as it enables controlled cultivation conditions to increase both biomass quality and product yield (Pomponi 1999, Sipkema et al. 2005, Pomponi 2013). Yet, while cell lines have been successfully established from various terrestrial invertebrate taxa (e.g. insects, nematodes), until

recently, no stable cell line had been developed for any marine invertebrate and all efforts (including in our laboratories) to obtain lasting proliferating cultures had inexplicably failed (Rinkevich 1999, Rinkevich 2005, Pomponi 2006, Grasele et al. 2011, Pomponi 2013). Seven reviews on marine invertebrate cell cultures (Bayne 1998, Rinkevich 1999, Mothersill and Austin 2000, Rinkevich 2005, Rinkevich 2011, Cai and Zhang 2014, Domart-Coulon and Blanchoud 2022) (covering over 250 peer-reviewed publications), have revealed that most research in this area has prioritised applied aspects of cell cultures (e.g. ecotoxicology, parasitology), over the development of innovative cell culture methodologies. Consequently, detailed knowledge of *in vitro* methodologies and approaches for developing long-lasting cell proliferation from marine invertebrates are missing.

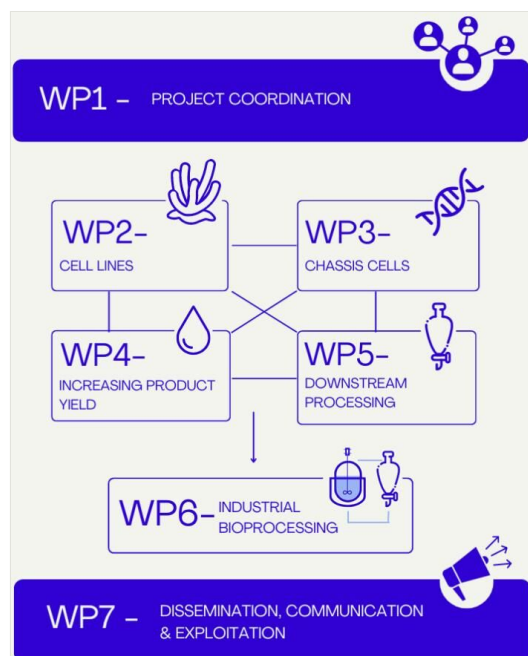


Figure 1.

Graphical representation of the BLUES Work Package structure and interdependencies.

Marine Invertebrate Cell Lines

A key motivation of the BLUES consortium is to develop reliable methodologies for continuously proliferating cell cultures from marine invertebrates. This development is based on an earlier EU Horizon 2020 grant agreement (No. 679849), where the first marine invertebrate (sponge) cell line that exhibited rapid cell doubling rates (more than 100 population doublings) was established (Hesp et al. 2023). Primary cell cultures of the sponge *Axinella corrugata* were previously shown to produce the bioactive compound stevensine (Andrade et al. 1999). These two accomplishments mark a critical starting

point for bioprocess development, demonstrating the potential of sponge cells for sustained metabolite production under controlled conditions at TRL3. Further studies are, however, required to explore continuous metabolite production in cell lines derived from other sponge species, assessing yields compared to those in whole organisms; identify additional metabolites that can be produced, and determine whether the yield can be maximised by varying cell culture conditions. Additionally, we recently evaluated the responses of different blood cell types from *Botryllus* sp. (a model colonial tunicate) to *in vitro* conditions, using five distinct formulations of cell culture media (Andrade et al. 1999). This study contributes to broader initiatives aimed at creating cell type-specific cultures and underscores the importance of optimised nutrient media and matrices for developing cell cultures and selecting specific cell types.

Table 2. Species and metabolites of interest for the BLUES project. Additional species from each of the four phyla may also be explored, depending on sample availability and time constraints.

Phylum	Species	Metabolite(s) of interest
Porifera	<i>Geodia barretti</i> (134023) <i>Axinella corrugata</i> (165472)	Barettins (11177588)/Barretides Stevensine (11003581)
Cnidaria	<i>Stylophora pistillata</i> (206982)	Mycosporine-like amino acids
Echinodermata	<i>Parastichopus tremulus</i> (124535) <i>Cucumaria frondosa</i> (124612)	Bioactive peptides/ Fucoidan (92023653)/ Saponins (6540709) Frondoside (44448161)/ Collagen
Chordata	<i>Botryllus schlosseri</i> (103862)	Botryllin

In line with this objective, WP2 is dedicated to developing primary cell cultures and establishing continuously dividing cell lines from four phyla of marine invertebrates: Porifera (sponges), Cnidaria (corals), Echinodermata (sea cucumbers) and Chordata (ascidians) (Table 2). This work is conducted in parallel with the activities in WP3 (development of chassis cells to produce any marine invertebrate compound using hybridoma technology and development of chassis cells to produce a specific compound from cell cultures of the producing organism) and is closely linked with WP4 (development of 3D cultures). The results from WP2 will feed directly into the optimisation of downstream processing (WP5) and industrial bioprocessing (WP6).

Novel Extraction Methodologies for Secondary Metabolites

Secondary metabolites extracted from wild-harvested marine invertebrates are typically present in low concentrations. These low yields make extraction unsustainable, as the biomass required would place significant pressure on natural populations, threatening species' viability. This creates a harmful cycle in which increasing demand leads to

overharvesting, further endangering marine biodiversity. Moreover, variability in metabolite content due to environmental factors makes wild harvesting an unreliable and inconsistent source for large-scale production.

Deep eutectic solvents (DESs) have emerged as promising green solvents, gaining considerable attention in both academia and industry in recent years due to their low toxicity, non-volatility, low vapour pressure, high boiling point and ease of preparation. DES are typically formed through mixtures of Bronsted or Lewis acids and bases, usually involving at least one hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), resulting in a eutectic system (Fig. 2). By varying the type and ratio of HBD and HBA components, DES can be tailored to exhibit specific physicochemical properties, making them highly versatile for targeted applications. The particularly notable subclass of natural deep eutectic solvents (NaDES) (Duan et al. 2016) are composed of naturally occurring primary metabolites that play an important role in cellular processes (e.g. sugars, carboxylic acids, amino acids and alcohols). These components are generally abundant and non-toxic, rendering NaDES both environmentally sustainable and safe for use in food and pharmaceutical extractions. Recently, DESs have been explored as alternative solvents for extraction of bioactive compounds, offering a “greener” substitute for conventional organic solvents. They have shown potential in the extraction of a variety of metabolites (e.g. flavonoids, proteins, lipids and polysaccharides) from sources like plants, microalgae and lignocelluloses (Duan et al. 2016). Due to their highly tunable nature, DESs also open the door to integrated, multi-product biorefinery process, thereby increasing the sustainability of the extraction processes.

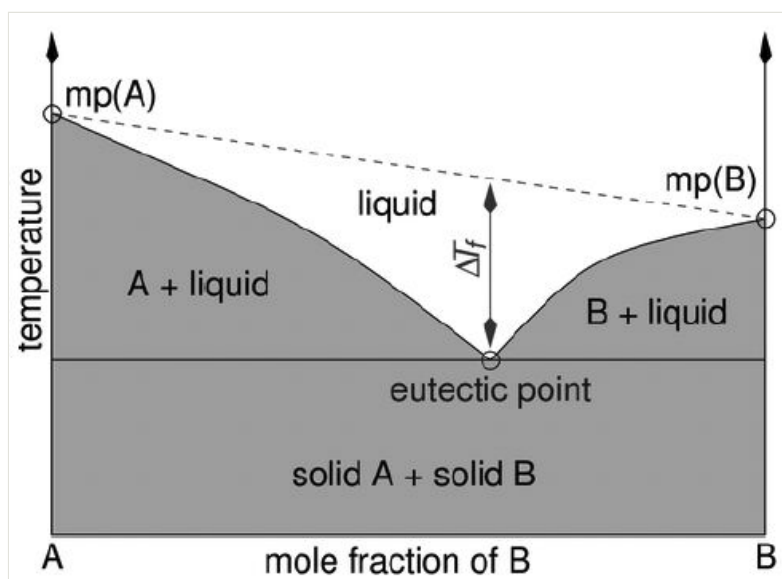


Figure 2.

Schematic representation of a eutectic point on a two-component phase diagram (Smith et al. 2014).

Recently, we developed a complete extraction process using DES for isolating lipids from the microalga *Nannochloropsis oceanica* (Lo et al. 2021), alginate and pigments from Brown seaweed (Hiemstra et al. 2025, Hiemstra et al. 2026, Reynaga-Navarro et al. 2026). Within WP5, the focus is on advancing downstream processing methods; an often overlooked yet critical aspect of marine biotechnology. Given the typically low concentrations of target metabolites in marine biomass, conventional methods often have a significant environmental footprint and generate substantial waste. To address this, WP5 aims to develop mild, efficient and environmentally sustainable extraction protocols that not only reduce the ecological impact, but also enable a multi-product approach. By thoroughly exploring the composition of each cell line, WP5 seeks to recover multiple valuable compounds without compromising their quality, ensuring that co-products remain intact and suitable for high-value applications. This integrated strategy maximises the use of marine biomass, improves overall resource efficiency and enhances the economic viability of marine bioprocessing.

Increasing Product Yields

As noted earlier, extraction yields of marine-derived metabolites are often low. The BLUES project addresses this challenge through two key strategies: increasing product yields and intensifying the overall production process. To enhance metabolite yields, several approaches will be implemented, including the optimisation of culture media tailored to species-specific needs, investigating the genetic mechanisms underlying metabolite biosynthesis, the use of hybridoma technology and exploring co-culture systems involving invertebrate hosts and their algal or microbial symbionts.

One major strategy for culturing sponge cells has involved adapting existing mammalian cell culture media by supplementing them with salts to mimic sea water (formulations such as Marine M199) and incorporating additional components, such as growth factors to stimulate cell proliferation (Pomponi 1997, Pomponi et al. 1998a, Pomponi et al. 1998b , Willoughby and Pomponi 2000, Krasko et al. 2001, Zhang et al. 2002). Recently, we optimised amino acid concentrations, based on metabolic activities for the sponge *Dysidea etheria*, leading to the development of a new medium, called 'M1' (Munroe et al. 2019). Following this, we observed rapid cell division in nine additional sponge species (Conkling et al. 2019). In particular, we established finite cell lines for three species of the same genus, *Geodia* cf. *gibberosa*, *G. neptuni* and *G. barretti*, which could be subcultured up to five times and achieved a maximum of seven population doublings (Conkling et al. 2019). Building on this, we formulated an enhanced medium, OpM1, containing growth factors and other stimulatory ingredients, which enabled the creation of the first continuous marine sponge cell line from *G. barretti*, achieving nearly 100 population doublings (Hesp et al. 2023). The results also demonstrated that sponge cells are capable of extremely rapid division. *G. barretti* cells exhibited a doubling time of under one hour in M1 and under 30 minutes in OpM1 (Hesp et al. 2023). For WP2 activities, previously successful media (Zhang et al. 2002) will serve as the starting point for new experiments aimed at achieving multiple cell passages in medium-throughput cell-culture systems using well plates, with further validation in flask-based cultures or bioreactors.

The effectiveness of *in vitro* conditions will be assessed, based on increases in cell numbers, cell proliferation rates and culture longevity.

We further investigated the *in vitro* responses of various blood cell types from *Botryllus* (a model colonial tunicate), using five distinct formulations of cell culture media (Andrade et al. 1999). This study supports broader initiatives to develop cell type-specific cultures and highlights the critical role of tailored nutrient media in promoting cell cultures and selecting for specific cell types. For WP2 activities, this targeted approach will be further pursued and extended to coral cell culture. For echinoderms, media optimisation will involve the use of growth media supplemented with sea cucumber derived extracellular matrix (ECM) proteins and the development of an animal-component-free liquid medium suitable for both suspension and adherent cultures of sea cucumber cells. Insights gained from these efforts may also be applied to refine and optimise culture conditions for sponges, corals and ascidians.

Understanding the relationship between gene expression patterns, specifically, genes that are up- or down-regulated and the production of bioactive compounds is key to deciphering the complex biosynthetic pathways involved. This knowledge can provide information for strategies to enhance metabolite yields and clarify whether these compounds originate from the host invertebrate, its microbial symbionts or both (Munroe et al. 2019). In WP3 activities, one of the tasks is to understand the genetic mechanisms underlying both cell immortalisation and metabolite biosynthesis in marine invertebrates, as well as to explore the potential for genetic modification in these cell lines. To this end, cells will be characterised at each passage, focusing on telomerase activity and immortality markers (e.g. mortalin, survivin, klf13, Ajelp2). In addition, multi-passaged epithelial cell cultures derived from the body walls of sea cucumbers will be studied for the expression of genes associated with the biosynthesis of selected bioactive peptides, fucoidan and saponins (antimicrobial, anti-inflammatory and anti-cancer compounds with established potential) (Atanassova et al. 2024). These analyses will be conducted as part of a broader whole-expression gene ontology study. The most promising cell lines will then be tested under various culture conditions to assess: (a) changes in bioactive peptide, fucoidan and saponin synthesis and (b) shifts in immortality marker expression, allowing for the identification of optimal candidate cell lines for further development.

WP2 will also explore the development of co-culture systems of coral cell with algal symbionts as well as sponge cell with microbial symbionts. The underlying rationale is that co-cultivation with symbionts may enhance the longevity and proliferation of invertebrate cell cultures. In the case of corals, maintaining a functional association with symbiotic algae could support sustained cell growth by boosting biomass and promoting continuous cell division. These algae, which reside within the coral's endodermal cells, provide a major portion of the host's energy requirements through the translocation of photosynthetically fixed carbon, while the coral cells actively regulate the physiology of their symbionts, creating a mutually beneficial interaction (Radax et al. 2012).

In the case of the sponge *Geodia barretti*, it is believed that barrettins are produced either through the symbiotic relationship between the sponge and its associated microbes or by

the microbial symbionts alone (Steffen et al. 2021). After several passages of *G. barretti* cells, baretins were no longer detectable (partner WU, unpublished), suggesting that culture conditions, while favourable for sponge cell divisions, may have led to the loss of essential microbial symbionts. To test this hypothesis, cells from selected *G. barretti* passages will be analysed to determine shifts or reductions in microbial community composition, with the aim of identifying microbiome members that may be critical for sponge cell growth and/or production of baretins. Cultures will be analysed using 18S and 16S rRNA gene sequencing from different passages, alongside chemical analyses of the same samples for the presence of the baretins and the barrettides (the latter being sponge-derived compounds (Steffen et al. 2021)). Based on the findings, culture conditions will be optimised, particularly through the change or removal of antibiotics in the media, to support microbiome maintenance. Further, metagenomic analyses will be performed to define potential co-metabolic pathways involved in metabolite biosynthesis.

In situations where bioactive compounds originate from species whose cells cannot be cultured or where biosynthetic pathways are unknown or difficult to engineer, hybridoma technology offers a viable alternative for developing production strains. Traditionally used to generate monoclonal antibodies by fusing antibody-producing B cells with rapidly dividing tumour cells, this approach creates cells (hybridomas) that combine high productivity with continuous cell division (Pomponi et al. 2013). A similar strategy can be applied by fusing rapidly dividing marine invertebrate cells with cells capable of producing target compounds, potentially yielding hybrid cell lines that inherit both traits (Fig. 3). We have already demonstrated the successful fusion of cells from different marine sponge species (Pomponi 2013), highlighting them as promising candidates for generating sponge-based hybridomas.

As part of WP3, *Geodia barretti* cells will be fused with compound-producing marine invertebrate cells that currently lack the ability to divide in culture (Fig. 4). The resulting hybridomas will be screened for their ability to produce the target compound(s). Potential fusion partners include cells from corals, sea cucumbers, ascidians and other sponges, whose culture conditions are being optimised under WP2.

Process Intensification

Process intensification involves enhancing the productivity of a given process or output, ultimately leading to greater efficiency and improved sustainability. Within BLUES, process intensification is a core objective integrated across all work packages. To maximise cell density and biomaterial yields, culture conditions must be carefully tailored to each species and target product. Key parameters requiring optimisation include vessel type, temperature, oxygen and carbon dioxide concentrations, pH and agitation.

Bioreactor Design

Most biopharmaceuticals, including monoclonal antibodies produced in CHO cells, are manufactured in large (up to 25,000 litres) stirred-tank bioreactors (Pérez-López et al.

2017). These systems are ideal for suspension cell cultures, which are readily scalable, well mixed and have a high gas transfer capacity through sparging air/oxygen bubbles. One of the key objectives of BLUES is to test the feasibility of using suspension cultures for each of the target marine invertebrate species. Our preliminary findings from sponge cells cultures indicate that, despite being anchorage-dependent, sponge cells are capable of proliferating in suspension. Until these cell suspension cultures are fully optimised or proven unfeasible, cells will also be grown in bioreactor systems that support anchorage-dependent growth. WP2 and WP6 will assess a range of bioreactor configurations. Stirred-tank reactors, for instance, can accommodate suspension cells or support adherent cells directly by using microcarrier beads (Sieblist et al. 2015, Yeshanew et al. 2016, Pérez-López et al. 2017, Tripathi and Shrivastava 2019). Alternatively, hollow-fibre reactors (Wung et al. 2014) allow cells to attach to the exterior of semi-permeable fibres, while nutrients flow through the interior, offering continuous nutrient delivery and product collection, an ideal setup for slow-growing, adherent cell lines. Small-scale testing of these different reactor types will provide information for the selection of optimal systems for scaling-up sponge cells cultures. WP2 and WP4 are integrating efforts to determine the attachment dependency of sponge cells and testing lab-scale bioreactors suitable for suspension cultures.

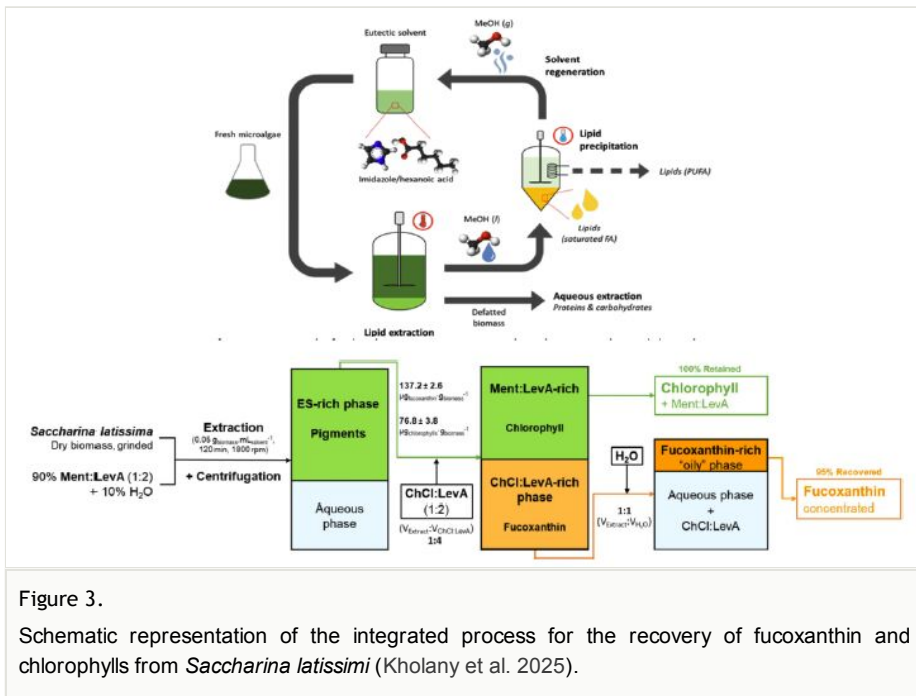


Figure 3.

Schematic representation of the integrated process for the recovery of fucoxanthin and chlorophylls from *Saccharina latissima* (Kholany et al. 2025).

Three-Dimensional (3D) Cell Culture

Two-dimensional (2D) cell culture remains a widely used, reliable and reproducible method for studying cellular behaviour. However, 2D systems fall short in replicating the

structural and functional complexity of *in vivo* environments. They lack the 3D microarchitecture and the dynamic cellular interactions present within a biologically active extracellular matrix. In contrast, 3D cultures provide a more physiologically relevant environment, allowing cells to interact with each other and with the surrounding matrix, simulating mechanical and biochemical stimuli found in living tissues (Cacciamali et al. 2022). The potential of 3D cell culture has been extensively explored in human tissue engineering and regenerative medicine, where biomaterials are used as 3D scaffolds to support or even guide cellular growth and tissue development, ultimately aiming to restore lost tissue function (O'Brien 2011, Campuzano and Pelling 2019). These 3D structures have been developed from a variety of materials, including natural or synthetic, polymers or ceramics, designed to mimic the native extracellular matrix (ECM) of the target species' tissue. Amongst the natural-origins, marine organisms have emerged in recent years as promising sources, with key advantages for human medicine, as, unlike mammalian-derived products, marine-derived compounds pose fewer risks on compatibility with the human body, allergic reactions, zoonotic transmission and ethical or religious constraints (Silva et al. 2012, Fassini et al. 2021).

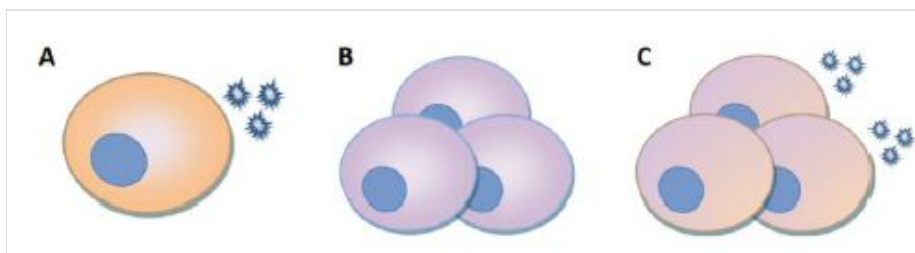


Figure 4.

Illustrating sponge hybridoma principle. Uncultivable sponge cells that produce compounds of interest, simplified by the asterisks (A) can be fused with a sponge cell line from another species (B) to create a hybrid cell line (C) that inherits both traits, immortality and bioactive compound production (Cai and Zhang 2014).

Within the scope of the marine invertebrate groups studied in this project, various materials have been investigated for 3D scaffold development. From sponges, various compounds, such as bio-silica (Wang et al. 2014), collagen/spongins (Swatschek et al. 2002) and chitin (Mutsenko et al. 2017), have been utilised to fabricate porous scaffolds and hydrogels. The naturally porous and interconnected architecture of sponge skeletons has also been highlighted as ideal tissue engineering templates (Tsurkan et al. 2020). For echinoderms, research has focused on mutable collagenous tissues and bio-adhesives, including collagen extracted from sea urchins, which has been used to produce membranes and 3D scaffolds for seeding mammalian cells (Fassini et al. 2021). These add to the many other marine origin materials being widely explored in biotechnology, as chitosan, fish collagen/gelatin and polysaccharides from seaweeds (namely alginate), which can act as pivotal materials for the generation of template matrices for 3D cell culture (Silva et al. 2012).

The aggregation and proliferation of sponge cells are mediated by their interaction with specific components of the ECM, as well as with biominerals (Pozzolini et al. 2014) and defined substrates (Pozzolini et al. 2009). Identifying these components and understanding their roles in cell interaction is a key element for optimising cell culture conditions that replicate the *in vitro* microenvironments in 3D systems, thereby enabling large-scale sponge cell growth and differentiation. For example, the ECM in sponges is composed of a variety of components, including proteins, polysaccharides and biominerals. Amongst the protein constituents, collagens and spongin are predominant. While sponge collagens share similarities with those found in terrestrial animals, they also exhibit unique structural features (Pozzolini et al. 2011). Their terrestrial counterparts have demonstrated strong potential as biomaterials for cell culture applications (Soroushanova et al. 2019). These biomaterials have already been evaluated for use in tissue engineering with mammalian cells, showing promising results in terms of biocompatibility and mechanical strength (Pozzolini et al. 2018). Due to their unique properties and compatibility with mammalian cells, marine sponge ECM components are considered excellent candidates for developing innovative scaffolds or enhancing bioreactor systems aiming at sustainable marine invertebrate cell cultures (Pozzolini et al. 2021). Leveraging expertise in biomaterials development, BLUES partners aim to create 3D scaffolds from marine-derived materials (e.g. sponge and sea cucumber collagens and ECMs), that support the cultivation of invertebrate cells capable of producing industrially valuable metabolites. Furthermore, functionalising these scaffolds with biochemical cues specific to the targeted marine invertebrate species will generate better performing constructs.

The objectives of WP4 are to replicate *in vitro* the native expression of invertebrate metabolites by focusing on several key areas, including molecular characterisation of the extracellular matrix from the targeted invertebrate species (primarily sponges and echinoderms), development of 3D cell culture matrices using widely available and sustainable marine-derived materials, enhancement of these matrices through the incorporation of ECM-specific components tailored to each taxon and functionalisation of the 3D templates with invertebrate cell growth factors to promote optimal cell proliferation and metabolite expression.

Industrial Bioprocessing

Building on the outcomes from WP2-5, WP6 will focus on developing bioprocess designs for scale-up of metabolite production for BLUES's species of interest. These designs will incorporate flowsheets and detailed mass and energy balances covering production, extraction and the recirculation of growth media and solvents. For one selected phylum, process feasibility will be demonstrated at TRL5. A model-based approach will guide experimental design, with results feeding back into the model, to refine and optimise the process, thus applying an iterative Design-Build-Test-Learn methodology. This will begin at the laboratory scale, followed by biomass production and extraction in small-scale pilot setups.

The resulting process designs developed in WP6 will be used to assess scalability through scenario analysis developed at various production scales. These scenarios will provide information for a techno-economic assessment that considers not only mass and energy flows, but also cost factors to evaluate economic viability and identify potential economic bottlenecks. Identifying these constraints will help establish research targets for future development. This analysis will not only be done on the economic aspects, but further on environmental sustainability such as energy and water consumption and CO₂ footprint.

The foundational principles of marine invertebrate cell line cultures have only recently been uncovered. BLUES advances this emerging field by deepening the understanding of the underlying molecular and biological mechanisms, which will drive improvements in both processes and end-products. Beyond this, BLUES extends industrial-scale process design to additional target species, laying the groundwork for the development of new production chassis for unique and complex marine metabolites. The project moves beyond basic research by integrating these insights with scalable production technologies, efficient product recovery and the recirculation of side streams. This integrated approach will provide information for the creation of a strategic roadmap to guide the continued advancement and implementation of innovations in blue biotechnology.

Concluding Remarks

The BLUES project is set to transform the production of marine-derived compounds by replacing traditional wild harvesting with sustainable, *in vitro* cultivation techniques. This shift not only safeguards marine biodiversity, but also empowers the blue bioeconomy to develop valuable natural products for healthcare and industrial use. With a multidisciplinary team of experts, the project is well-positioned to deliver high-impact research and promote meaningful collaboration.

In addition to its scientific advancements, BLUES is committed to educating marine professionals and informing global policy-makers. By raising public awareness about marine conservation and connecting science with policy and society, the project supports both environmental stewardship and the advancement of health-related innovation.

Author Contributions

Conceptualisation, Methodology and Writing have been done by all authors according to their background. All authors have read and approved the final text.

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Conflicts of interest

The authors have declared that no competing interests exist.

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